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# Crystallization and preliminary X-ray study of α-glucosidase from *Geobacillus* sp. strain HTA-462, one of the deepest sea bacteria

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An α-glucosidase (EC 3.2.1.20) was purified from *Geobacillus* sp. strain HTA-462 cells and crystallized using the hanging-drop vapour-diffusion technique. The *Geobacillus* strain is a thermophilic and high-pressure-resistant bacterium found at the bottom of the Challenger Deep in the Mariana Trench. The crystal was characterized by X-ray diffraction and belongs to space group *C*2, with unit-cell parameters $a = 104.0$, $b = 91.5$, $c = 72.9$ Å, $\beta = 109.4^\circ$. Diffraction data to 2.5 Å resolution were collected and processed.

## 1. Introduction

*Geobacillus* sp. strain HTA-462 is a microorganism that was found in a sediment sample collected by the unmanned submarine Kaiko from the bottom of the Challenger Deep in the Mariana Trench at a depth of 10 897 m (Takami *et al.*, 1997). Strain HTA-462, one of several thousand Mariana isolates, showed thermophilic (328–348 K) and high-pressure-resistant (∼30 MPa) growth patterns. Based on a 16S rRNA sequence comparison (Takami *et al.*, 1997), this isolate was classified as a member of the genus *Geobacillus*, which comprises mostly thermophilic bacteria, including *G. stearothermophilus* (formerly *Bacillus stearothermophilus*) as the type species (Nazina *et al.*, 2001).

The deep-sea frontier is expected to be a field that will provide new biological resources such as enzymes with extremophilic characters. *Geobacillus* sp. strain HTA-462 was found to produce an α-glucosidase (EC 3.2.1.20; Hung *et al.*, personal communication). α-Glucosidase hydrolyzes the 1,4- or 1,6-bonds of α-D-glucoside at the non-reducing end in an exoglycolytic manner (Sinnott, 1991; McCarter & Withers, 1994). Because α-glucosidases also catalyze the transglycosylation reaction of the α-D-glucose moiety, the enzymes have potential use in the industrial biosynthesis of oligosaccharides or glycoconjugates (Kurimoto *et al.*, 1997; Mala *et al.*, 1999).

The α-glucosidase extracted from *Geobacillus* sp. strain HTA-462 cells is a protein with a molecular mass of 60 kDa (as determined by SDS–PAGE). The enzyme shows optimal activity under alkaline (pH 9.0) and high-temperature (333 K) conditions. It efficiently catalyzes the transglycosylation reaction with maltose as the sugar donor. The enzyme can use some non-sugar molecules, such as chloramphenicol, cortisone and alcohols, as sugar acceptors (Hung *et al.*, personal communication).

The thermostability and the wide substrate specificity of the enzyme indicate a high potential for industrial applications. In order to obtain structural details of this enzyme, crystallization and preliminary X-ray diffraction experiments were carried out.

## 2. Materials and methods

HTA-462 α-glucosidase was used for the crystal-growth experiment. The bacterium was propagated, with shaking, for 1 d at 323 K in a liquid medium composed of 2%(*w*/*v*) Polypepton S (Wako Pure Chemical), 0.5%(*w*/*v*) meat extract (Wako Pure Chemical), 0.05%(*w*/*v*) yeast extract (Difco), 0.1%(*w*/*v*) $KH_2PO_4$, 0.02%(*w*/*v*) $MgSO_4$ and 1.0%(*w*/*v*) soluble starch (Nakarai Chemical). The HTA-462 α-glucosidase was purified to homogeneity with a yield of 30% by a several-step procedure involving column chromatography on DEAE-Toyopearl (Tosoh), hydroxyapatite (Seikagaku Kogyo), phenyl-Toyopearl (Tosoh), and Bio-Gel A-1.5m (Tosoh) columns using phosphate buffer pH 7.0 for the equilibration of the columns and the elution of proteins. The purified enzyme was dialyzed overnight against 2 l of 10 m*M* phosphate buffer pH 7.0 and was concentrated to 1%(*w*/*w*) on a PM-10 membrane (10 kDa cutoff, Amicon). The protein concentration was measured using a Bio-Rad protein assay kit with bovine serum albumin as a standard.

Crystallization conditions for the HTA-462 α-glucosidase were set up with the hanging-drop vapour-diffusion method using the Crystal Screen I and II reagents (Hampton Research). Crystals were obtained from initial conditions consisting of a 1 ml reservoir of 20%(*v*/*v*) 2-propanol, 20%(*w*/*v*) PEG 4000 in 0.1 *M* sodium citrate buffer pH 5.6 and a drop consisting of a mixture of 4 µl of the reservoir solution, 5 µl 1%(*w*/*v*) protein solution (the concentrated gel filtrate) and 1 µl 50 m*M*

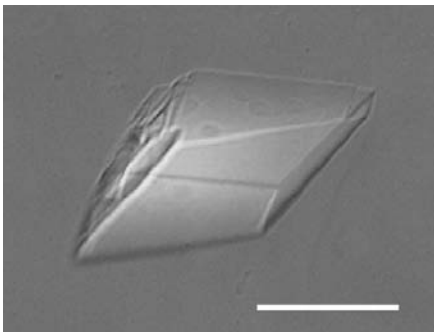

**Figure 1**
A crystal of HTA-462 α-glucosidase. The scale bar indicates ∼0.1 mm.

**Table 1**
Crystallographic parameters and data-collection statistics.

Values in parentheses refer to the reflections in the outermost shell.

| | |
|---|---|
| Space group | *C*2 |
| Unit-cell parameters | |
| *a* (Å) | 104.0 |
| *b* (Å) | 91.5 |
| *c* (Å) | 72.9 |
| β (°) | 109.4 |
| Resolution limits (Å) | 8.00–2.50 (2.59–2.50) |
| No. of observed reflections | 61656 (4122) |
| No. of unique reflections | 20149 (1850) |
| Redundancy | 3.1 (2.2) |
| Average $I/\sigma(I)$ | 23.3 (8.6) |
| Completeness (%) | 93.0 (86.4) |
| $R_{\rm merge}$ (%) | 7.9 (16.5) |

D-(+)-glucono-1,5-lactone solution. D-(+)-Glucono-1,5-lactone is an inhibitor of α-glucosidase. Rhombohedral shaped crystals grew at 291 K to approximate maximum dimensions of 0.3 × 0.2 × 0.1 mm in 2–3 weeks (Fig. 1).

X-ray data were collected using an R-AXIS IV imaging-plate detector with a mirror monochromator mounted on a Rigaku RU-300 copper rotating-anode X-ray generator (Cu $K\alpha$; $\lambda$ = 1.5418 Å; Rigaku). The crystal was scooped up with a 0.2 mm nylon loop (Hampton Research) and flash-frozen in a liquid-nitrogen bath. Before freezing, the crystal was transferred to a 10 µl drop of reservoir solution containing 20%(*v*/*v*) glycerol. The crystal was kept in a 100 K dry nitrogen stream from an Oxford Cryosystems Cryostream during data collection. Diffraction data were recorded on imaging plates. Data processing took place using the *DENZO* and *SCALEPACK* programs (Otwinowski & Minor, 1997).

## 3. Results and discussion

The α-glucosidase from *Geobacillus* sp. strain HTA-462 was crystallized and diffraction data to 2.5 Å resolution were obtained. The crystal belongs to the monoclinic *C*2 space group, with unit-cell parameters $a$ = 104.0, $b$ = 91.5, $c$ = 72.9 Å, $\beta = 109.4^\circ$. The data-processing statistics are shown in Table 1. The asymmetric unit of the crystal probably contains one protein molecule with an expected $V_{\rm M}$ = 2.6 Å$^3$ Da$^{-1}$, which corresponds to a solvent content of ∼53% (Matthews, 1968).

Although the inhibitor of the enzyme, D-(+)-glucono-1,5-lactone, was used as an additive for the crystal from which the present data were collected, it was found that the same crystals could also be grown under the same conditions without the inhibitor. No appreciable difference in size and quality was observed between the crystals grown with or without the inhibitor (data not shown).

As the cloning and sequencing of the gene for the HTA-462 α-glucosidase is still in progress, the position of this enzyme in the glycoside hydrolase classification system (Henrissat & Bairoch, 1996) has not yet been identified. At this point in time, it is uncertain whether or not the molecular-replacement method could be used to solve the crystal structure. Assuming the HTA-462 α-glucosidase to be a representative of a novel family, a search for heavy-atom derivatives for MIR phasing is currently in progress.

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